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Forns index and fatty liver index, but not FIB-4, are associated with indices of glycaemia, pre-diabetes and type 2 diabetes: analysis of The Maastricht Study

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ABSTRACT

Objective Glucose metabolism status (GMS) is linked to non-alcoholic fatty liver disease (NAFLD). Higher levels of advanced glycation end products (AGEs) are observed in people with type 2 diabetes mellitus (T2DM) and NAFLD. We examined the association between GMS, non-invasive tests and AGEs, with liver steatosis and fibrosis.

Methods Data from The Maastricht Study, a population-based cohort, were analysed. Participants with alcohol overconsumption or missing data were excluded. GMS was determined via an oral glucose tolerance test. AGEs, measured by skin autofluorescence (SAF), were assessed using an AGE Reader. Associations of GMS and SAF with the fibrosis-4 score (FIB-4), Forns index (FI) and fatty liver index (FLI) were investigated using multivariable linear regression, adjusted for sociodemographic, lifestyle and clinical variables.

Results 1955 participants (56.6%) were analysed: 598 (30.6%) had T2DM, 264 (13.5%) had pre-diabetes and 1069 (54.7%) had normal glucose metabolism. Pre-diabetes was significantly associated with FLI (standardised regression coefficient (Stβ) 0.396, 95% CI 0.323 to 0.471) and FI (Stβ 0.145, 95% CI 0.059 to 0.232) but not FIB-4. T2DM was significantly associated with FLI (Stβ 0.623, 95% CI 0.552 to 0.694) and FI (Stβ 0.307, 95% CI 0.226 to 0.388) but not FIB-4. SAF was significantly associated with FLI (Stβ 0.083, 95% CI 0.036 to 0.129), FI (Stβ 0.106, 95% CI 0.069 to 0.143) and FIB-4 (Stβ 0.087, 95% CI 0.037 to 0.137).

Conclusion The study showed that adverse GMS and higher glycaemia are positively associated with steatosis. FI, but not FIB-4, was related to adverse GMS concerning fibrosis. This study is the first to demonstrate that SAF is positively associated with steatosis and fibrosis.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ NAFLD is closely linked to T2DM and prediabetes, with elevated risks of liver steatosis and fibrosis in these populations.

WHAT THIS STUDY ADDS

⇒ Unlike FIB-4, FI is linked to liver fibrosis in T2DM, and SAF is associated with both steatosis and fibrosis, indicating potential as an early screening tool.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

⇒ These findings could prompt further investigation into FI and SAF as primary screening tools for NAFLD, leading to new guidelines for managing NAFLD in diabetes clinics.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a condition defined by the presence of steatosis in more than 5% of the hepatocytes in people without secondary causes for steatosis (eg, drugs or alcohol). NAFLD is an umbrella term for a spectrum of liver disorders, including non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), which can progress to fibrosis and cirrhosis with or without decompensation and hepatocellular carcinoma.[1 2] NAFLD is the most common liver disorder in the Western world, with a current prevalence of 32.4% in the general population.[3] The prevalence is expected to continue to increase worldwide in line with the epidemic proportions of obesity, type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVDs).[4–6] The most widely accepted pathophysiological mechanisms relating T2DM to NAFLD include central obesity and insulin resistance (IR).[7 8] Long-standing expansion of the adipose tissue in people with obesity causes the recruitment

of proinflammatory cells, leading to low-grade inflammation and, eventually, IR.[9 10] Moreover, the association between T2DM and NAFLD appears bidirectional in that NAFLD increases the risk of T2DM up to two times, and T2DM increases the risk of NAFLD by 2–6 times.[11 12]

Several non-invasive test scores (NITs), used as surrogate markers for steatosis or fibrosis risk determination, exist next to liver biopsy as the gold standard.[13] Multiple guidelines indicated the fibrosis-4 score (FIB-4) as the first step in screening for NAFLD-related fibrosis.[14–16] Nevertheless, Graupera *et al* indicated that the FIB-4 had a low accuracy when used in a general population.[17] As for risk groups such as T2DM, FIB-4 also showed a low accuracy in diagnosing advanced fibrosis.[18 19] The Forns index (FI), another NIT to assess fibrosis, has not been thoroughly investigated in an at-risk population, though it was validated in a NAFLD biopsy-proven cohort.[20] The fatty liver index (FLI) and T2DM have been closely associated, though little research has been performed to assess the association with other aspects of the glucose metabolism.[21] Associative studies to assess the relationship between NITs and the glucose metabolism might help to understand why NITs do not always perform equally well in different cohorts. We, therefore, explored the association between glucose metabolism status (GMS), steatosis and fibrosis as assessed with the different NITs within The Maastricht Study cohort population.

In addition, the association between IR, as measured by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) or the Matsuda Index, and the presence of steatosis and fibrosis was evaluated. We also investigated whether skin autofluorescence (SAF), fasting plasma glucose (FPG), 2-hour FPG (2h-FPG) and haemoglobin A1c (HbA1c) would be determinants of steatosis and fibrosis, measured with the surrogate markers FIB-4, FI and FLI, in this specific group. More specifically, we were interested in the relationship between SAF and steatosis or fibrosis. SAF measures the accumulation of advanced glycation end products (AGEs) in the skin that results from hyperglycaemia[22] and can be easily and non-invasively measured with an AGE reader.[23 24] Hence, this study aimed to examine the associations of GMS, IR, indices of glycaemia and SAF with liver steatosis, estimated from FLI and fibrosis, assessed from FIB-4 and FI, using well-characterised data from The Maastricht Study population-based cohort study.

METHODS

Study design and population

Data from The Maastricht Study, a prospectively designed, population-based observational cohort study, were used. The rationale and methodology have been described previously.[25] In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of T2DM and is characterised by an extensive phenotyping approach. All individuals aged between 40 and 75 years and living in the southern part of the Netherlands were eligible for participation. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status, with an oversampling of individuals with T2DM for reasons of efficiency.[25] The present report includes cross-sectional data from 3451 participants who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of 3 months.[25]

Glucose metabolism status

After an overnight fast, participants, except those who used insulin or had an FPG concentration above 11.0 mmol/L, underwent an oral glucose tolerance test (OGTT) postingestion of a 75 g glucose drink. Based on FPG, 2h-FPG and glucose-lowering medication use, GMS was determined as normal glucose metabolism (NGM), pre-diabetes (impaired fasting glucose, impaired glucose tolerance or both) or T2DM in accordance with the WHO 2006 criteria.[26]

Measures of glycemia

FPG and HbA1 were determined in venous plasma samples after an overnight fast. 2h-FPG was determined in venous plasma collected at 120 min postglucose drink ingestion. AGEs were assessed with the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands). The AGE reader uses the characteristic fluorescent properties of certain AGEs to quantify their accumulation in the skin as SAF.[27] The AGE Reader illuminates a skin surface of 4 cm^2, shielded from other light and uses the ratio of the reflection of fluorescent light (wavelength 420–600 nm) to non-fluorescent light (300–420 nm) to calculate SAF.

Calculation of non-invasive scores

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, albumin, triglycerides, total cholesterol and gamma-glutamyl transpeptidase were determined in venous blood samples. Anthropometric measurements were performed to determine body mass index (BMI) and waist circumference. Age at the time of the measurements was used. Non-invasive scores to assess the risk for steatosis (FLI) and fibrosis (FIB-4 and FI) were calculated (online supplemental table S1).[28–30]

Covariates

We assessed educational level, smoking status, alcohol use and history of CVD by questionnaires.[25] Dietary habits, including adherence to the Dutch Healthy Diet index,[31] were based on a validated food frequency questionnaire.[32] Medication use was assessed during a medication interview. 24-hour ambulatory blood pressure (BP) was measured, and total daily physical activity was measured with an accelerometer.[33] The lipid profile and IR (estimated by the HOMA2 model or Matsuda Index) were determined in fasting venous blood, post-glucose load and OGTT samples.

Table 1 Overview of the regression models used to investigate the association between the non-invasive scores, GMS and measures of glycaemia

	FLI	FIB-4	FI
Model 1	Crude	Crude	Crude
Model 2	Age, sex and educational status	Sex and educational status	Sex and educational status
Model 3	Model 2 with office systolic BP, antihypertensive medication, smoking status, lipid-modifying medication, history of CVD and glucose-lowering medication	Model 2 with office systolic BP, antihypertensive medication, total cholesterol/HDL ratio, smoking status, lipid-modifying medication, history of CVD and glucose-lowering medication	Model 2 with office systolic BP, antihypertensive medication, smoking status, lipid-modifying medication, history of CVD and glucose-lowering medication
Model 4	Model 3 with total cholesterol/HDL ratio	Model 3 with waist circumference	Model 3 with waist circumference
Model 5	Model 3 with IR	Model 4 with IR	Model 4 with total cholesterol/HDL ratio
Model 6	Model 3 with total cholesterol and IR	N.A.	Model 3 with IR

BP, blood pressure; CVD, cardiovascular diseases; FI, Forns index; FIB-4, fibrosis-4 index; FLI, fatty liver index; GMS, glucose metabolism status; HDL, high-density lipoprotein; IR, insulin resistance.

Statistical analysis

Multivariable linear regression analyses were used to investigate the associations of GMS (dummy variables of pre-diabetes or T2DM vs NGM) and standardised FPG, 2h-FPG, HbA1c and SAF with standardised FLI, FIB-4 and FI.

Non-invasive scores

Table 1 serves as an overview of the models used to investigate the associations between the non-invasive scores (FLI, FIB-4 and FI), GMS and the measures of glycaemia. For the FLI, we left waist circumference out of the model since it is an element of the FLI score. Similarly, for the FIB-4 and FI, age was left out of the models since it is an element of both the FIB-4 and FI. The following models were chosen as the main models and used in describing the results: model 3 for FLI, and for the FIB-4 and FI, model 4 will be used. The other models were used to study the influence of total cholesterol/high-density lipoprotein ratio, waist circumference and IR on the regression analysis.

Additional analyses

We repeated the analyses with additional adjustments for lifestyle factors added to the main models of each regression analysis (dietary score, physical activity level). Second, we replaced waist circumference with BMI; educational status with occupational status or income level and office systolic BP with office diastolic BP, systolic or diastolic 24-hour ambulatory BP; and HOMA-IR index with the Matsuda Index. The Matsuda Index indicates both hepatic and peripheral insulin sensitivity while the HOMA-IR describes glucose-insulin homoeostasis. Third, an interaction analysis was conducted to assess the influence of sex on the regression models, and if significant, the associations were stratified by sex.

All analyses were performed with Statistical Package for Social Sciences V.28.0 (IBM SPSS, IBM). For all analyses, a $p<0.05$ was considered statistically significant. Categoric variables were presented as percentages with absolute values. The Shapiro-Wilk test was used to test for normality of numerous variables. All skewed numeric variables are presented as median with percentiles. The associations were expressed as standardised regression coefficient (Stβ) and corresponding 95% CI. Collinearity diagnostics were used to detect excessive multicollinearity between covariates.

RESULTS

Selection and characteristics of the study population

Figure 1 gives an overview of the study population selection. Participants with excessive alcohol abuse (>2 glasses/day for women and >3 glasses/day for men) or missing data on the NITs, GMS and confounders were excluded (n=1496).

The median age was 60 (53–66), 63 (57–69), 62 (56–67) and 58 (51–64) years for the total study cohort (n=1955) and people with T2DM (n=598), pre-diabetes (n=264) and NGM (n=1069), respectively (table 2 and figure 1). Overall, people with T2DM had a higher BMI and waist circumference, less physical activity and a lower diet score compared with the other two groups ($p<0.005$) (data not shown). The general characteristics of participants, except for a higher age and a slightly lower BMI, included in the study were comparable to those excluded (online supplemental table S2).

GMS and non-invasive scores

Table 3 shows the associations between GMS and liver steatosis or fibrosis. After full adjustment, a more adverse GMS was associated with a higher FLI (model 3 (table 3); standardised beta (95% CI), T2DM versus

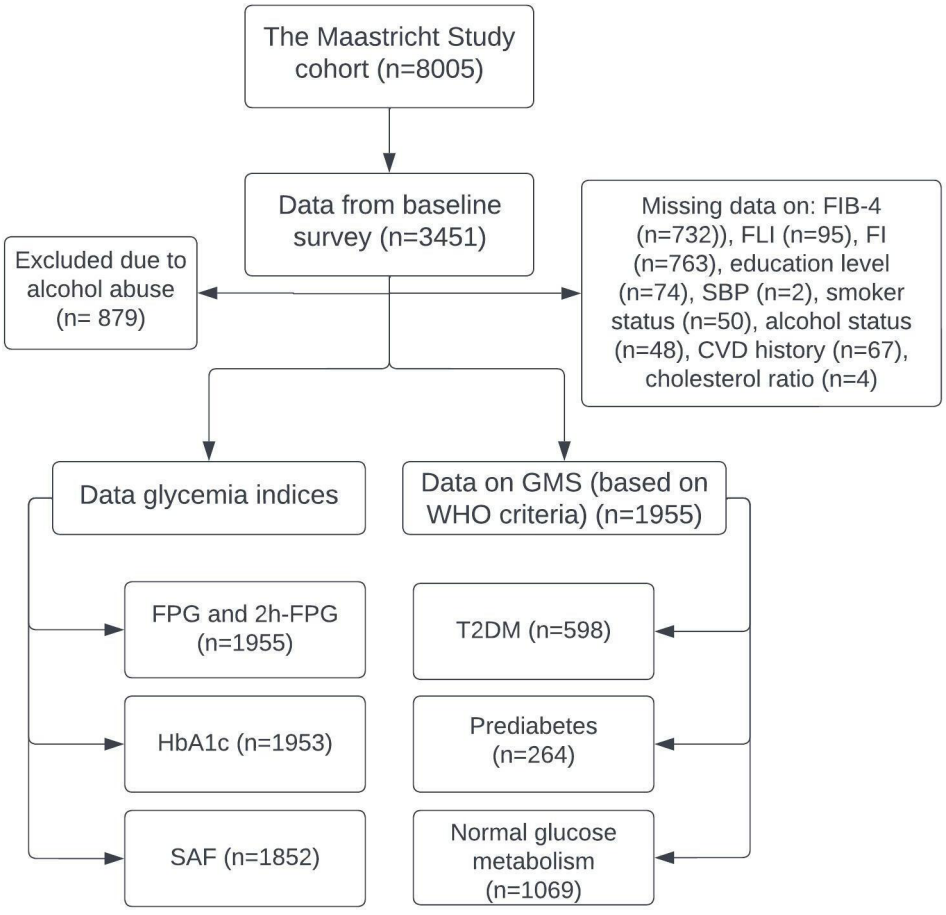

Figure 1 Overview of study population selection. CVD, cardiovascular disease; FI, Forns index; FIB-4, fibrosis-4; FLI, fatty liver index; FPG, fasting plasma glucose; GMS, glucose metabolism status; HbA1c, haemoglobin A1c; SAF, skin autofluorescence; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

NGM 0.829 (0.728; 0.929) with p for trend <0.001 and pre-diabetes versus NGM 0.503 (0.391; 0.615) with p for trend <0.001). This was also seen for the FI, where T2DM versus NGM, but not pre-diabetes, was associated with greater FI (model 4 (table 3); 0.203 (0.106; 0.300) with p<0.001). For the FIB-4, a significant negative association (table 3; −0.136 (−0.264; −0.009) with p=0.036) was found for T2DM in model 4. An association between FIB-4 and pre-diabetes was not found.

Association between FPG, 2h-FPG, HbA1c and SAF, and non-invasive scores

Table 4 shows the association between FLI, FI and FIB-4 and the other glycaemia indices. After full adjustment (model 3), it was seen that higher indices of glycaemia were significantly associated with a higher FLI, with Stβ of 0.307 (0.261; 0.353) for FPG; 0.284 (0.240; 0.329) for 2h-PG; 0.267 (0.221; 0.314) for HbA1c and 0.083 (0.036; 0.129) for SAF. FPG, 2h-FPG and SAF showed modest significant positive associations with the FI (per SD, 0.049 (0.011; 0.088), 0.108 (0.066; 0.149), 0.106 (0.069; 0.143), respectively). No significant association was found between HbA1c and FI (model 4). Next, for the FIB-4 (model 4), a low positive association was found for SAF (per SD, 0.087 (0.037; 0.137)) but not for the other indices of glycaemia. For FPG and HbA1c, a slight significant negative association was seen (per SD, −0.067 (−0.124; −0.010) and −0.106 (−0.163; −0.049)), and for 2h-FPG, no association was found.

Interaction analysis

The interaction analysis showed that sex modified several associations (online supplemental table S3). Sex modified the associations of T2DM (p value interaction=0.027) and SAF (p=0.025) with FIB-4. Also, the associations between T2DM, FPG and HbA1c with FLI (p=0.002, p=0.016, p=0.002, respectively) were modified by sex. Additional analysis identified a significant inverse association in females for the FIB-4 T2DM association (−0.144 (−0.039; −0.541)) but not in males (online supplemental table S4). For the SAF, the male sex showed a significant positive association (0.125 (0.061; 0.189)). Next, sex modified the FLI associations with T2DM, with stronger associations for males (0.567 (0.450; 0.684)) than for females (0.415 (0.342; 0.487)). Similar trends were observed for FPG (with 0.176 (0.129; 0.222) for males and 0.123 (0.093; 0.154) for females) and for HbA1c (0.149 (0.101;0.197) male vs 0.052 (0.022;0.082) female).

Additional analysis

The regression model with the addition of the lifestyle factors, namely diet score and physical activity, showed

Table 2 Descriptive statistics of study population with complete data on glucose metabolism status

Characteristics	Total population (n=1955)	T2DM patients (n=598)	Pre-diabetes (n=264)	NGM (n=1069)
Age (years)	60 (53–66)	63 (57–69)	62 (56–67)	58 (51–64)
Sex (% male)	1030 (52.7)	389 (65.1)	143 (54.2)	485 (45.4)
BMI (kg/m^2)	26.5 (24.1–29.8)	29.6 (26.3–32.9)	27.3 (24.8–30.2)	25.3 (23.1–27.5)
Waist circumference (cm)	95.3 (86.4–104.5)	104.5 (96.2–114.3)	98.4 (91–105)	90.0 (82.8–98)
Office systolic blood pressure (mm Hg)	134 (122–146)	140 (129–151)	136 (126–148)	129 (118–142)
Office diastolic blood pressure (mm Hg)	76 (69–83)	77 (71–83)	78 (71–84)	75 (68–82)
Smoking status				
Never	764 (39.1)	189 (31.6)	92 (34.8)	478 (44.7)
Former	950 (48.6)	322 (53.8)	144 (54.5)	471 (44.1)
Current	241 (12.3)	87 (14.5)	28 (10.6)	120 (11.2)
Alcohol consumption				
None	486 (24.9)	224 (37.5)	60 (22.7)	197 (18.4)
Low (women ≤20 g, men ≤30g/day)	1469 (75.1)	374 (62.5)	204 (77.3)	872 (81.6)
Educational level and lifestyle				
Low	706 (36.1)	290 (48.5)	105 (39.8)	300 (28.11)
Intermediate	574 (29.4)	169 (28.3)	77 (29.2)	321 (30.0)
High	675 (34.5)	139 (23.2)	82 (31.1)	448 (41.9)
Physical activity (hours/day)	1.9 (1.5–2.4)	1.6 (1.1–2.1)	1.9 (1.5–2.4)	2.1 (1.7–2.5)
Diet score	85.1 (74.9–95.3)	82.3 (71.2–90.7)	84.2 (75.–95.1)	87.4 (77.1–97.2)
Medical background and medication use				
Use of antihypertensive medication (%)	830 (42.5)	435 (72.7)	122 (46.2)	260 (24.3)
History of cardiovascular disease (%)	340 (17.4)	163 (27.3)	32 (12.1)	141 (13.2)
Use of lipid-lowering medication (%)	738 (37.7)	450 (75.3)	90 (34.1)	184 (17.2)
Use of glucose-lowering medication (%)	515 (26.3)	491 (82.1)	0 (0.0)	0 (0.0)
Laboratory values				
Thrombocytes in whole blood (10×10^9/L)	238.0 (203.0–276.0)	239.0 (194.0–276.3)	232.0 (202.5–271.5)	238.0 (205.0–277.0)
ALT (U/L)	26.0 (21.0–34.0)	31.0 (23.0–43.0)	28.0 (23.0–35.0)	24.0 (20.0–31.0)
AST (U/L)	26.0 (22.0–32.0)	27.0 (22.0–34.0)	26.0 (22.0–33.0)	25.0 (22.0–31.0)
Serum albumin (g/L)	44.6 (42.7–46.2)	44.8 (42.5–46.5)	44.5 (42.8–46.2)	44.7 (42.9–46.0)
Serum triglycerides (mmol/L)	1.2 (0.9–1.7)	1.5 (1.1–2.1)	1.3 (1.0–1.8)	1.1 (0.8–1.4)
GGT (U/L)	23.0 (17.0–36.0)	30.0 (22.0–47.0)	25.5 (18.0–37.0)	20.0 (15.0–29.0)
Total-to-HDL cholesterol ratio	3.4 (2.8–4.2)	3.5 (2.8–4.2)	3.5 (3.0–4.4)	3.3 (2.8–4.1)
Measures of glycaemia				
Fasting plasma glucose (mmol/L)	5.5 (5.0–6.7)	7.5 (6.7–8.7)	6.0 (5.4–6.3)	5.1 (4.8–5.5)
2-hour post-load glucose (mmol/L)	6.3 (5.1–9.9)	14.7 (12.1–17.5)	8.5 (7.8–9.5)	5.4 (4.6–6.2)
HbA1c (%)	5.6 (5.4–6.3)	6.5 (6.3–7.5)	5.6 (5.4–5.9)	5.4 (5.2–5.6)
Skin autofluorescence (AU)	2.2 (1.9–2.5)	2.4 (2.1–2.7)	2.2 (1.9–2.5)	2.1 (1.8–2.4)
Insulin resistance (estimated by the HOMA2 model)	1.4 (1.0–2.2)	2.1 (1.4–3.2)	1.7 (1.2–2.5)	1.2 (0.9–1.7)
Non-invasive scores				
FIB-4 score	1.3 (1.0–1.7)	1.3 (1.0–1.8)	1.3 (1.0–1.7)	1.3 (1.0–1.6)
Forns index	4.5 (3.6–5.5)	5.3 (4.5–6.3)	4.6 (3.7–5.4)	4.0 (3.4–4.9)
Fatty liver index	45.7 (20.5–74.0)	76.4 (51.4–91.6)	53.9 (30.5–77.8)	29.5 (13.6–52.3)

Data are presented as mean±SD, median (IQR) or number (%).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; AU, arbitrary units; BMI, body mass index; FIB-4, fibrosis-4 index; GGT, gamma-glutamyl transpeptidase; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; HOMA, Homeostatic Model Assessment; NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

Table 3 Associations of glucose metabolism status with indicators of liver steatosis and fibrosis

	FLI		FI		FIB-4	
	Stβ (95% CI)	**P value**	**Stβ (95% CI)**	**P value**	**Stβ (95% CI)**	**P value**
Pre-diabetes vs NGM						
Model 1 (Crude)	0.662 (0.505; 0.739)	<0.001	0.325 (0.201; 0.449)	<0.001	0.098 (–0.035; 0.232)	0.150
Model 2	0.556 (0.444; 0.669)	<0.001	0.230 (0.119; 0.342)	<0.001	0.064 (–0.069; 0.198)	0.343
Model 3	**0.503 (0.391; 0.615)**	**<0.001**	0.086 (–0.018; 0.190)	0.104	0.023 (–0.112; 0.157)	0.741
Model 4	0.412 (0.310; 0.514)	<0.001	**0.070 (–0.035; 0.175)**	**0.190**	**0.033 (–0.103; 0.168)**	**0.633**
Model 5	0.298 (0.200; 0.396)	<0.001	0.096 (–0.005; 0.198)	0.063	0.034 (–0.101; 0.170)	0.620
Model 6	0.275 (0.183; 0.367)	<0.001	0.057 (–0.050; 0.163)	0.296	N.A.	N.A.
T2DM vs NGM						
Model 1 (crude)	1.112 (1.023; 1.200)	<0.001	0.893 (0.801; 0.985)	<0.001	0.070 (–0.032; 0.172)	0.178
Model 2	0.972 (0.883; 1.062)	<0.001	0.691 (0.605; 0.777)	<0.001	0.000 (–0.105; 0.105)	0.996
Model 3	**0.829 (0.728; 0.929)**	**<0.001**	0.235 (0.142; 0.327)	<0.001	–0.156 (–0.278; –0.034)	0.012
Model 4	0.719 (0.627; 0.810)	<0.001	**0.203 (0.106; 0.300)**	**<0.001**	**–0.136 (–0.264; –0.009)**	**0.036**
Model 5	0.460 (0.369; 0.551)	<0.001	0.220 (0.125; 0.314)	<0.001	–0.133 (–0.262; –0.005)	0.042
Model 6	0.457 (0.372; 0.542)	<0.001	0.182 (0.081; 0.283)	<0.001	N.A.	N.A.

Main models are highlighted in bold.
Model 1: crude results. Model 2: sex and educational status. Model 3: model 2 with office systolic BP, use of antihypertensive medication, total cholesterol/HDL cholesterol ratio (only for FIB-4), lipid-modifying medication, smoking status, history of cardiovascular disease and use of glucose-lowering medication. Model 4: model 3 with waist circumference for the FIB-4 and FI, for the FLI with total cholesterol/HDL ratio. Model 5: model 4 adjusted with IR for FIB-4 and FLI (without total cholesterol/HDL ratio for FLI) and with total cholesterol/HDL ratio for the FI. Model 6: all confounders for FLI and FI with IR adjustment. Not included in all the models of the FIB-4 and FI is age. Waist circumference was not included in the FLI. SD: FLI±29.84, FI±1.38 and FIB4±0.76.
BP, blood pressure; FI, Forns index; FIB-4, fibrosis 4 score; FLI, fatty liver index; HDL, high-density lipoprotein; IR, insulin resistance; N.A., not applicable; NGM, normal glucose metabolism; Stβ, standardised beta; T2DM, type 2 diabetes mellitus.

Table 4 Associations for the indices of glycaemia FPG, 2h-FPG, HbA1c and SAF with indicators of liver steatosis and fibrosis

	FLI		FI		FIB-4	
	Stβ (95% CI)	P value	Stβ (95% CI)	P value	Stβ (95% CI)	P value
Fasting plasma glucose, per SD						
Model 1 (crude)	0.499 (0.455; 0.544)	<0.001	0.325 (0.283; 0.367)	<0.001	0.025 (–0.025; 0.074)	0.330
Model 2	0.412 (0.368; 0.457)	<0.001	0.219 (0.181; 0.258)	<0.001	–0.018 (–0.069; 0.033)	0.481
Model 3	**0.307 (0.261; 0.353)**	**<0.001**	0.064 (0.026; 0.101)	<0.001	–0.075 (–0.130; –0.020)	0.008
Model 4	0.260 (0.218; 0.301)	<0.001	**0.049 (0.011; 0.088)**	**0.012**	**–0.067 (–0.124; –0.010)**	**0.021**
Model 5	0.119 (0.076; 0.162)	<0.001	0.051 (0.013; 0.088)	0.008	–0.067 (–0.127; –0.008)	0.025
Model 6	0.114 (0.073; 0.154)	<0.001	0.049 (0.003; 0.096)	0.037	N.A.	N.A.
2-hour post-load glucose, per SD						
Model 1 (Crude)	0.420 (0.378; 0.462)	<0.001	0.354 (0.0311; 0.397)	<0.001	0.057 (0.010; 0.103)	0.017
Model 2	0.371 (0.329; 0.412)	<0.001	0.290 (0.251; 0.329)	<0.001	0.036 (–0.011; 0.084)	0.135
Model 3	**0.284 (0.240; 0.329)**	**<0.001**	0.119 (0.079; 0.159)	<0.001	–0.021 (–0.074; 0.032)	0.441
Model 4	0.237 (0.196; 0.278)	<0.001	**0.108 (0.066; 0.149)**	**<0.001**	**–0.016 (–0.071; 0.038)**	**0.556**
Model 5	0.155 (0.116; 0.194)	<0.001	0.199 (0.079; 0.159)	<0.001	–0.017 (–0.072; 0.038)	0.540
Model 6	0.138 (0.102; 0.174)	<0.001	0.094 (0.051; 0.137)	<0.001	N.A.	N.A.
HbA1c, per SD						
Model 1 (crude)	0.442 (0.398; 0.487)	<0.001	0.281 (0.239; 0.324)	<0.001	–0.011 (–0.066; 0.024)	0.653
Model 2	0.374 (0.330; 0.417)	<0.001	0.197 (0.158; 0.236)	<0.001	–0.043 (–0.093; –0.006)	0.087
Model 3	**0.267 (0.221; 0.314)**	**<0.001**	0.013 (–0.025; 0; 052)	0.502	–0.111 (–0.166; –0.056)	<0.001
Model 4	0.219 (0.177; 0.262)	<0.001	**–0.005 (–0.044; 0.035)**	**0.823**	**–0.106 (–0.163; –0.049)**	**<0.001**
Model 5	0.148 (0.106; 0.189)	<0.001	–0.002 (–0.040; 0.037)	0.925	–0.105 (–0.162; –0.049)	<0.001
Model 6	0.131 (0.092; 0.169)	<0.001	–0.001 (–0.046; 0.044)	0.966	N.A.	N.A.
SAF, per SD						
Model 1 (crude)	0.202 (0.156; 0.248)	<0.001	0.279 (0.235; 0.323)	<0.001	0.123 (0.076; 0.169)	<0.001
Model 2	0.139 (0.091; 0.186)	<0.001	0.215 (0.176; 0.255)	<0.001	0.106 (0.059; 0.153)	<0.001
Model 3	**0.083 (0.036; 0.129)**	**<0.001**	0.110 (0.073; 0.147)	<0.001	0.083 (0.034; 0.132)	0.001
Model 4	0.087 (0.046; 0.129)	<0.001	**0.106 (0.069; 0.143)**	**<0.001**	**0.087 (0.037; 0.137)**	**<0.001**
Model 5	0.052 (0.013; 0.090)	0.009	0.097 (0.061; 0.133)	<0.001	0.087 (0.037; 0.136	<0.001
Model 6	0.063 (0.026; 0.099)	<0.001	0.106 0.068; 0.145)	<0.001	N.A.	N.A.

Main models are highlighted in bold.
Model 1: crude results. Model 2: sex and educational status. Model 3: model 2 with office systolic BP, use of antihypertensive medication, total cholesterol/HDL cholesterol ratio (only for FIB-4), lipid-modifying medication, smoking status, history of cardiovascular disease and use of glucose-lowering medication. Model 4: model 3 with waist circumference for the FIB-4 and FI, for the FLI with total cholesterol/HDL ratio. Model 5: model 4 adjusted with IR for FIB-4 and FLI (without total cholesterol/HDL ratio for FLI) and with total cholesterol/HDL ratio for the FI. Model 6: all confounders for FLI and FI with IR adjustment. Not included in all the models of the FIB-4 and FI is age. Waist circumference was not included for the FLI. SD: FLI±29.84, FI±1.38 and FIB4±0.76.
BP, blood pressure; FI, Forns index; FIB-4, fibrosis 4 score; FLI, fatty liver index; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; IR, insulin resistance; N.A., not applicable; NGM, normal glucose metabolism; SAF, skin autofluorescence; Stβ, standardised beta; T2DM, type 2 diabetes mellitus.

overall similar results when compared with the models without the lifestyle factors. A significant, positive association for FLI, GMS and all indices of glycaemia was found (online supplemental table S5). As for the FI, significant positive associations were found for T2DM, 2h-FPG and SAF. Lastly, the FIB-4 was negatively associated with FPG, HbA1c, and T2DM and positively with SAF. Quantitatively similar results were observed in the performed sensitivity analysis for all dependent variables (online supplemental table S6). When using the Matsuda Index instead of the HOMA-IR, analogous quantitative results were seen (online supplemental table S7).

DISCUSSION

The present population-based study indicates that liver steatosis is positively associated with GMS as determined by the non-invasive serum marker FLI. Having prediabetes and T2DM implicates a higher FLI and, thus, a higher risk of having NAFLD. Also, FPG, 2h-FPG and

HbA1c were associated with FLI. As determined by the non-invasive serum marker FI, liver fibrosis was positively associated with T2DM, FPG and 2h-FPG. Next, although a non-invasive marker of fibrosis like the FI, no associations were seen between FIB-4, pre-diabetes and 2h-FPG. Moreover, a negative association was found with T2DM, FPG and HbA1c. As for SAF, we found that it was positively associated with all non-invasive serum markers, FLI, FIB-4 and FI. Finally, we observed that NAFLD may have been driven by IR, as seen in the FLI models, where we see a decline in the coefficients when IR was added. Notwithstanding, for the fibrosis models, the coefficients stayed similar when adding IR.

IR drives the pathogenesis of NAFLD in the first stages of the disease.[13] Adipose tissue expands due to the sedentary lifestyles and high caloric intake, triggering adipose tissue IR and inflammation.[34 35] Together with the increased production of reactive oxygen species (ROS) caused by mitochondrial uncoupling, dysfunctional adipose tissue and endotoxins from the gut, a proinflammatory climate in the liver is promoted, leading to NASH.[36–38] If the proinflammatory climate persists, it ultimately leads to tissue injury and scar tissue formation of the liver (fibrosis).[39] Fibrosis is thus not directly driven by IR, as is reflected by our results.

Our findings concerning steatosis, GMS and the other indices of glycaemia align with observations from previous research.[40] Notwithstanding, it is important to recognise that also pre-diabetes is linked to a higher FLI, as it has been seen that the coexistence of NAFLD and pre-diabetes has an additive effect on the risk of developing T2DM.[41] Although a surrogate marker of steatosis, FLI can thus be used as an early and non-invasive predictor of incident diabetes among people with pre-diabetes.[42 43] As for the other indices of glycaemia, we found that FPG and 2h-FPG were linked to steatosis. FPG is known as an important risk factor for diabetes and CVD.[44] However, three Asian studies also named FPG a possible risk factor for NAFLD.[45–47] In a study by Li *et al*, the ratio of people with impaired fasting glucose increased with the grade of steatosis, indicating a link between one of the early markers of diabetes and steatosis.[48] As for HbA1c, two recent studies found a strong association with steatosis.[49 50]

We found that pre-diabetes was not associated with liver fibrosis. Although these findings were confirmed by a recent large cohort study from South Korea using magnetic resonance elastography,[51] other studies found the opposite.[52 53] Yilmaz *et al* showed a strong association between pre-diabetes and fibrosis in a small biopsy-proven NAFLD cohort.[53] The difference in sample size and diagnostics might explain the conflicting results. Additionally, although the association between FI and pre-diabetes was not significant due to a smaller sample size, it has the same direction as the association between FI and T2DM and is, therefore, clinically relevant. Regarding the association between fibrosis and T2DM, our findings align with previous literature.[54–56] A recent study using data from an extensive database in the USA indicated that T2DM increased the risk of fibrosis among individuals with obesity and NAFLD.[57] Moreover, our data showed that the FI, not the FIB-4, could be a potential marker for fibrosis among T2DM. The FIB-4 was initially developed for the detection of significant fibrosis in hepatitis C (HCV)/HIV positive people and contains the parameters AST and ALT in the formula.[30] People with an active HCV infection often have disturbed AST and ALT.[58] Though in the case of NAFLD, this is not always true.[59] The absence of a disturbed AST/ALT ratio could explain why the FIB-4 is not ideal for detecting NAFLD-related fibrosis, as was found by several other studies.[17 18 60] Next, we found that only FI, but not FIB-4, was positively linked to FPG and 2h-FPG. Fiorentino *et al* did not calculate the fibrosis scores; however, they did find a significant difference in liver enzymes AST and ALT when comparing groups with NGM or pre-diabetes stratified by 1h-FPG,[61] suggesting that a higher FPG or 2h-FPG might be linked to liver inflammation and possibly fibrosis. Lastly, HbA1c was not related to fibrosis. Two recent studies corroborated this with logistic regression analysis, showing no association between liver fibrosis and HbA1c.[49 50] In contrast, a large cross-sectional study conducted in Japan with 15 785 participants found that the HbA1c level in people without T2DM was associated with FIB-4.[62] The difference in sample size and ethnic background might explain the difference between our analysis and the Japanese study.

Our findings underscore potential sex differences that may influence the associations under investigation. Specifically, for the association with the FLI, sex modified the relationships with T2DM, FPG and HbA1c, suggesting that these associations are enhanced by both sexes, although potentially to varying degrees, with males having a stronger positive association than females. Next, we observed that in males, higher SAF levels were positively associated with the presence of T2DM. Lastly, we observed that a higher FIB-4 was less strongly associated with having T2DM in females than in males. These differences may be attributed to biological factors, variations in exposure to risk factors (eg, hazardous work environments) and health behaviours (eg, smoking or alcohol consumption) between sexes, as well as the protective effects of oestrogen during the reproductive age of women.[63] The latter might not be visible in our study cohort, where the majority of women were probably postmenopausal, given the age range of 40–75 years, possibly explaining sex neutrality on the association between FLI and glycaemic indices.[64] In contrast, in the association between FIB-4 and T2DM, the female sex appears to have a protective effect against developing fibrosis. Multiple studies have investigated the association between fibrosis and female sex, but it led to conflicting results, with some studies reporting a clear difference between premenopausal women who had a lower fibrosis severity when compared with men and postmenopausal women and other studies reporting no influence of sex on the risk of fibrosis development.[65]

In our cohort, SAF, the measure for AGEs accumulation, was positively associated with both surrogate markers for steatosis and fibrosis. Higher levels of AGEs have been observed not only among people with T2DM but also in normoglycaemic individuals with NAFLD.[66] Literature concerning NITs and their possible association with SAF is very limited. However, the CODAM study, which included people without diabetes and with (pre)diabetes, showed an association between circulating AGEs, liver fat, estimated by the FLI and low-grade inflammation.[67] Recent evidence indicated AGEs might be involved in the liver injury axis and pathogenesis of NAFLD.[67 68] Glyceraldehyde-derived (GA) AGEs, a type of AGEs able to be detected by fluorescence, are produced in the liver via different pathways, including fructolysis and glycolysis.[69] Increased consumption of both fructose and glucose in, for example, sugar-sweetened beverages is associated with NAFLD,[69–71] linking AGEs to NAFLD as is explained in the toxic AGE theory by Takeuchi *et al*.[69] The interaction between GA-AGEs and the receptor (RAGE) has been seen to alter intracellular signalling, gene expression, release of proinflammatory molecules and promotes oxidative stress with ROS generation,[72 73] leading to cytotoxicity in hepatic stellate cells and hepatocytes.[72 74] Moreover, AGEs have also been linked to vascular, renal and retinal complications in T2DM.[75–79]

Strengths of this study include the large size of this population-based cohort with oversampling of individuals with type 2 diabetes, which enabled accurate comparison of individuals with and without diabetes, in addition to the extensive phenotyping of the population with a vast number of potential confounders. Furthermore, a substantial number of potential confounders was considered, and an ample amount of sensitivity analyses were performed, which generally yielded consistent and, thus, robust study findings.

The study has several limitations. Given our cross-sectional design, causal relationships cannot be made on the investigated relationships.[80] In addition, a considerable number of people needed to be excluded due to missing data (n=265) or excessive alcohol use (n=879), leading to lower, though still sufficient, statistical power. The 2-hour post-load glucose results are most susceptible to this form of selection bias, as no data were available in individuals with diabetes treated with insulin because they were excluded from undergoing an OGTT. Such range restriction may lead to underestimated associations.[81] Third, a single OGTT may misclassify GMS due to day-to-day variability in glucose levels (impact of acute factors, eg, carbohydrate intake, level of physical activity), and it may not capture long-term glycaemic control. Therefore, individuals classified with pre-diabetes based on their first OGTT are relatively more prone to receive an NGM classification than when based on a second OGTT; this would likely lead to an underestimation of the association with the non-invasive scores in the pre-diabetes group.[82] On the other hand, classifying people as being prediabetic on the first OGTT while the second OGTT classifies them as having an NGM is also possible, leading to a bias towards the null and underestimating the current associations. Fourth, we cannot entirely exclude bias due to residual confounding factors (eg, environmental factors such as air pollution).[83 84] Nonetheless, we took an extensive set of confounders into account and conducted sensitivity analyses. Fifth, it is known that haemolysis could disturb AST and, in a lesser manner, ALT measurements.[85 86] However, no data were gathered on the haemolysis of blood samples during the study, though it is unlikely that a lot of haemolytic samples were present in the dataset as they usually cannot be assessed. The models with IR are an overadjustment as IR is a mediator. IR is part of both T2DM and NAFLD pathology. Next, SAF is an estimate of the concentration of AGEs. Nevertheless, SAF correlated well with the AGE concentration in skin biopsy samples and demonstrated usefulness in large-scale studies, like our cohort, to predict T2DM-related complications.[24 87] Another consideration is the usage of surrogate and not direct markers to estimate steatosis or fibrosis. As this was a large population-based cohort study, it was not feasible or ethical to submit each participant to a liver biopsy to determine the presence of steatosis and fibrosis. However, the FLI, FIB-4 and FI have been validated against liver biopsy results and showed good diagnostic performances.[28 88–92] Last, we studied Caucasian individuals aged 40–75 with access to high-quality care. Therefore, the generalisability of our results to other populations, especially outside of Europe, with different genetic, cultural and environmental health influences, requires further study. Moreover, the age range limits the relevance of our findings in younger individuals.

CONCLUSION

In summary, the present population-based study demonstrated that adverse GMS and higher glycaemia parameters are associated with liver steatosis, independent of demographics, cardiovascular risk factors and lifestyle risk factors. Concerning liver fibrosis, only FI and not the FIB-4 score showed a relation with adverse GMS. Furthermore, this study showed that SAF was positively associated with both steatosis and fibrosis. Future research is necessary to determine if the FI is an ideal marker for fibrosis in a cohort of people with (pre) diabetes. In addition, the value of SAF in detecting steatosis and fibrosis needs to be investigated thoroughly in an external cohort and more research is needed on FI and SAF as early non-invasive detectors in people with pre-diabetes. Finally, it is crucial to further explore the sex-based differences in the associations between fibrosis, steatosis and GMS.

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